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Mineral Distributions in Milling Fractions of Low Phytic Acid Wheat

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ABSTRACT

Low phytic acid (LPA) wheat (*Triticum aestivum* L.) is one approach to improving nutritional quality of wheat by reducing the major storage form of P and increasing the level of inorganic P (P_i), which is more readily absorbed by humans and other monogastric animals. A LPA mutant of wheat, designated Js-12-LPA was isolated following mutagenesis. LPA and wild-type (WT) sib selections of hard red spring wheat families with the pedigree 'Grandin'*4/Js-12-LPA were grown in replicated field trials in 2003 and 2004. Grain was milled on an experimental mill, and the distribution of P, phytic acid P (PAP), and P_i was measured in milling fractions. Mineral concentrations also were determined. LPA selections had elevated concentrations of P_i and Mg in flour fractions. The concentration of P_i in LPA flour was three times the concentration in WT flour, and Mg concentration in LPA flour was 25% greater than in WT flour. Therefore, P and Mg in LPA wheat appear to be redistributed within the kernel. The increase in P_i is similar to that observed for other LPA mutants and should improve the mineral nutrition of monogastric animals fed whole grain LPA wheat. As most wheat is milled for flour and bran, the detailed distribution of minerals in the LPA wheat should assist geneticists and nutritionists in assessing the value of this mutation.

Phytic acid (myo-inositol [1,2,3,4,5,6] hexa*kis*phosphate, abbreviated PA) is the major storage form of P in grain. We have identified a low phytic acid (LPA) mutant of wheat (Guttieri et al., 2004). Previous reports of LPA crops include barley (*Hordeum vulgare* L.; Larson et al., 1998), rice (*Oryza sativa* L.; Larson et al., 2000), soybean [*Glycine max* (L.) Merr.; Wilcox et al., 2000], and maize (*Zea mays* L.; Raboy et al., 2000).

Low phytic acid wheat is of interest as one approach to improving the nutritional quality of wheat fed to humans and livestock. Animals fed diets with LPA corn and barley have demonstrated greater feed efficiency, improved digestibility, better retention of P, Ca, and N, and significant decrease in P excretion (reviewed by Mendoza 2002). Human diets high in PA can lead to Zn deficiency, as PA is negatively correlated with Zn absorption. Phytic acid does not affect Cu absorption in humans, but slightly inhibits Mn absorption (reviewed by Lönnerdal 2002). PA forms insoluble complexes with Fe that are nutritionally unavailable at the pH of the small intestine. Diets high in PA and low in Fe can lead to Fe deficiency. However, in human populations with high Fe diets, the formation of PA–Fe complexes may provide protection against colon cancer by reducing Fe-induced oxidative injury (reviewed by Minihane and Rimbach 2002). Raboy et al. (1991) evaluated the quantitative relationship between grain PAP and total P in two winter wheat populations. Variation in PAP was observed, ranging from 30 to 48% of the population means. However, this variation in PAP was highly and positively correlated with variation in grain total P (r = 0.93 to 0.96). They did not observe redistribution of the storage form of P in the grain. To date, the only mechanism for altering the composition of P storage in wheat has been through the LPA mutant (Guttieri et al., 2004).

Phytic acid is accumulated in globoid bodies in the aleurone of wheat. Upon milling, PA concentration is highest in bran. For example, six Pakistani wheat cultivars were surveyed for PA and mineral content in whole wheat flour, bran, and straight grade flour (Anjum et al., 2002). (Straight grade flour is the flour that is produced after the bran and germ have been removed.) Phytic acid content of bran ranged from 4.2 to 6.1%; PA content of whole wheat flour ranged from 1.2 to 2.2%; and PA content of straight grade flour ranged from 0.2 to 0.5%. Mineral concentrations also were greatest in bran. Copper in bran ranged from 29 to 52 mg kg^{-1}, in straight grade flour from 5 to 12 mg kg^{-1}, and in whole wheat flour from 10 to 18 mg kg^{-1}. Iron concentration in bran, whole wheat flour, and straight grade flour ranged from 128 to 146 mg kg^{-1}, 46 to 99 mg kg^{-1}, and 26 to 46 mg kg^{-1}, respectively. Zinc concentration in bran, whole wheat, and straight grade flour ranged from 44 to 81 mg kg^{-1}, 21 to 28 mg kg^{-1}, and 9 to 34 mg kg^{-1}, respectively. Manganese similarly was of higher concentration in the bran.

As PA forms insoluble complexes with nutritionally important minerals, decreasing the PA/ P_i ratio may alter the distribution of minerals among bran and flour fractions. Our initial characterization of the Js-12-LPA wheat and its WT sib selection suggested that mineral distribution was altered among milling fractions of LPA wheat (Guttieri et al., 2004). However, the number of genotypes sampled (one WT and one LPA) and the number of environments evaluated (one) in the initial description of the LPA mutant, while appropriate for initial characterization of the mutant, were insufficient to describe the effects of the trait through time and space. In addition, the mutagenesis of Js-12 caused mutations to the selected line beyond the LPA trait, as evidenced by short stature, friable straw, and poor yield; the previous report did not attempt to isolate the effects of the LPA mutant from the background effects of the mutagenized Js-12-LPA line. A similar analysis was conducted by Bryant et al. (2005), which measured P and mineral concentrations in milled 'Kaybonnet' rice and a LPA mutant of Kaybonnet produced in unreplicated

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Abbreviations: HIP, high inorganic P; LPA, low phytic acid; PA, phytic acid; PAP, phytic acid P; P_i, inorganic P; WT, wild-type.

trials in 3 yr. A trend toward increased P, K, and Mg was observed in milled LPA rice relative to Kaybonnet.

The objective of the present study was to reach more generalized conclusions about the LPA mutant by characterizing the effect(s) of the LPA trait on mineral distribution in wheat milling fractions from a set of backcross-derived hard red spring sister selections grown in replicated field trials in 2 yr.

MATERIALS AND METHODS

Generation of Experimental Materials

Two families of F_2 plants derived from two BC_3F_1 plants with the pedigree 'Grandin'*4/Js-12 LPA were grown in the greenhouse. BC_3 F_1 plants were produced and progeny families (BC_3F_2) were derived from these plants. A preliminary evaluation of high inorganic P (HIP) phenotype to eliminate heterozygous genotypes was conducted on F_3 seed from each BC_3F_2 plant. High inorganic P phenotype of individual kernels was evaluated as described previously (Guttieri et al., 2004). The $BC_3F_{2:3}$ seed from BC_3F_2 plants determined to be homozygous for HIP phenotype or WT phenotype were hand-planted in the field near Aberdeen, ID, in 2002 with progeny rows tracing to each BC_3F_2 plant kept separate. Field-grown seed was evaluated for uniformity of HIP phenotype. Fourteen $BC_3F_{2:4}$ families were advanced into replicated yield trials in 2003, including three strong HIP phenotype LPA selections and three WT HIP selections derived from one BC_3 F_1 plant (designated as High LPA Family), and five moderate HIP phenotype LPA selections and three WT HIP selections from a second BC_3 F_1 plant (designated as Intermediate LPA Family). The designation of High and Intermediate LPA Family was confirmed by analysis of the replicated $BC_3F_{2:4}$ selections. All initial designations were validated in the progeny analysis.

Field Trials

$BC_3F_{2:4}$ selections, as well as the parents Grandin and Js-12- LPA, were grown in three environments using trials at the University of Idaho Aberdeen Research and Extension Center near Aberdeen, ID, in 2003 and 2004 and at the University of Idaho Tetonia Research and Extension Center near Tetonia, ID, in 2004. Trials were randomized arrangements of incomplete block designs in three replications. At Aberdeen, soil test P in the first 30 cm of soil was 26 mg kg^{-1} in 2003 and 13 mg kg^{-1} in 2004. At Tetonia, soil test P was 21 mg kg^{-1}. Plot size was 1.4 by 3 m. The experimental area was fertilized with ammonium nitrate before planting based on University of Idaho soil test recommendations. The experimental area at Aberdeen was irrigated using overhead sprinklers to replace estimated evapotranspiration. At Tetonia, the experimental area was not irrigated. Weeds were controlled with registered small grain herbicides per standard procedures. Trials at Aberdeen were harvested on 18 Aug. 2003 and 30 Aug. 2004 using a small plot combine equipped with a weighing system (Harvestmaster, Juniper Systems, Logan, UT). At Tetonia, trials were harvested on 23 Sept. 2004. Harvested grain was cleaned and test weight recorded.

Milling

Grain from each plot was tempered by the standard American Association of Cereal Chemistry (AACC 2000) method 26–10. Tempered grain was milled using a Brabender Quadrumat Senior Mill (Duisberg, Germany; AACC method 26–21A). Break flour is the flour recovered from the first mill roll; reduction flour is the flour recovered from the second mill roll. Bran is the unreduced fraction after the second mill roll. Shorts are fine bran sifted from the break and reduction flour fractions. Each of these four fractions were recovered separately for subsequent analysis.

Phosphorus Composition

Samples of bran, shorts, break flour, and reduction flour were dried in a 70°C drying oven for a minimum of 3 d before extraction or digestion so that P content could be expressed on a dry weight basis. Methods for analysis of P_i, total P, and PAP were as recently described (Guttieri et al., 2006). Briefly, P_i was determined colorimetrically by a variation of the Chen method (Chen et al., 1956) modified for use on microtiter plates. Total P was determined by digestion of dried samples in concentrated sulfuric acid and hydrogen peroxide followed by colorimetric detection. Phytic acid P was determined by modification of the colorimetric method of Haug and Lantzsch (1983). Each sample was plated in quadruplicate, and each experiment performed in duplicate.

Elemental Analysis

Mineral element composition of all milling fractions (bran, shorts, break flour, reduction flour) from grain produced at Aberdeen was determined by the University of Idaho Analytical Services Laboratory using a PerkinElmer Optima 3200 ICP–OES (Inductively Coupled Plasma–Optical Emission Spectrometer; PerkinElmer, Wellesley, MA) to quantify aqueous constituents following nitric acid digestion of the milling fractions. Mineral concentrations tested included Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, V, Zn, and Y. Mineral element composition of break flour from grain of one BC_3–derived family grown at Tetonia also was determined.

Statistical Analysis

Analyses of variance were conducted using a mixed model of fixed and random effects calculated with PROC MIXED in SAS (SAS Institute, 2000). Experiments initially were analyzed with year as a fixed effect to test the interaction of year with entry. Significant year × entry interactions were observed in all trials. Therefore years were analyzed separately. Data were analyzed with backcross family (B, C) and genotype (WT, LPA) as fixed effects and replication and family interaction with genotype within selection was treated as random effects.

RESULTS AND DISCUSSION

Whole Grain Phosphorus Distribution

Whole grain P concentration was unaffected by family or LPA genotype in all environments (Fig. 1, adapted from Guttieri et al., 2006). Total P concentrations in grain produced at Aberdeen were approximately 25% lower in 2004 than in 2003, possibly as a result of the 50% lower soil P concentration in the 2004 field. Phosphorus concentration in grain produced at Tetonia in 2004 was 20% lower than in grain produced in Aberdeen in 2004, although soil test P was intermediate between Aberdeen 2003 and 2004. The lower amount of applied irrigation at Tetonia may have reduced the P uptake from the soil system. In 2003 at Aberdeen, PAP accounted for 72% of the total P in the WT selections of the Intermediate LPA Family, and 64% of the total P in the WT

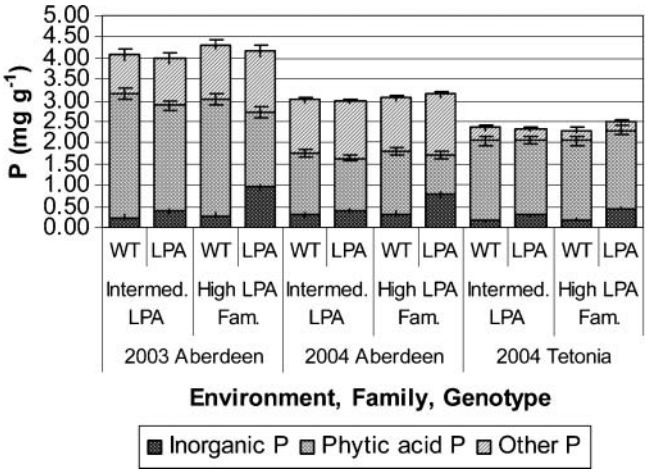

Fig. 1. Phosphorus distribution in whole grain of low phytic acid (LPA) and wild-type (WT) selections of two BC_3–derived families of the cross Grandin*4/Js-12-LPA. Grain was produced under irrigation at Aberdeen, ID, in 2003 and 2004 and under rain-fed conditions at Tetonia, ID, in 2004 (adapted from Guttieri et al., 2006).

selections of the High LPA Family. In 2004 at Aberdeen, when P concentrations in grain were lower, PAP accounted for only 48% of total P in grain of WT selections of both families. The LPA trait had a greater effect in the High LPA Family than in the Intermediate LPA Family in each of the three environments (Fig. 1).

Total Phosphorus Concentration in Milling Fractions

WT and LPA selections of both families milled similarly in both years of the study at Aberdeen. Averaged across families and genotypes, total flour yield (break + reduction) was 700 g kg^{-1} in both years. At Tetonia, total flour yield averaged 685 g kg^{-1}. Bran yield averaged 280 g kg^{-1}, and shorts yield averaged 20 g kg^{-1} at Aberdeen. In 2003, break flour yield averaged 15 g kg^{-1} and reduction flour yield 55 g kg^{-1}; in 2004, break flour yield averaged 17 g kg^{-1} and reduction flour yield averaged 53 g kg^{-1}.

Total P concentration in bran was not affected by genotype or family in any of the three production environments (Table 1). Total P concentration in bran was similar in both years of the study at Aberdeen, despite lower grain P concentration in 2004. The effect of the lower grain P concentration in 2004 was manifested in lower P concentration in break and reduction flour fractions. Total P concentration in bran was approximately 10 times greater than in flour. Total P concentration in shorts was about half the concentration in bran and, like the bran fraction, effects of genotype and family were limited. In 2003, concentration of P in the shorts from LPA grain was about 7% lower than in shorts from WT grain. Although we did not have a second year of data for Tetonia to provide similar yearly contrasts for that

Table 1. Total P concentration in milling fractions of grain grown at Aberdeen, ID, under irrigation in 2003 and 2004 and at Tetonia, ID, under rain-fed conditions in 2004. Concentrations are presented as the mean ± standard error.

	Total P concentration					
	Bran			Shorts		
Genotype†	2003 Aberdeen	2004 Aberdeen	2004 Tetonia	2003 Aberdeen	2004 Aberdeen	2004 Tetonia
	mg g^{-1}					
Intermediate LPA Family						
Wild-type (N = 3)	11.35 ± 1.11	11.25 ± 0.30	5.92 ± 0.31	6.48 ± 0.22	5.61 ± 0.32	3.17 ± 0.22
LPA (N = 5)	9.71 ± 1.08	10.22 ± 0.23	6.19 ± 0.24	6.04 ± 0.17	5.83 ± 0.30	3.26 ± 0.20
High LPA Family						
Wild-type (N = 3)	10.56 ± 1.11	11.02 ± 0.30	5.85 ± 0.31	6.31 ± 0.22	5.76 ± 0.32	3.07 ± 0.22
LPA (N = 3)	10.61 ± 1.12	10.84 ± 0.30	6.61 ± 0.31	5.84 ± 0.23	5.79 ± 0.32	3.31 ± 0.22
	Mixed effects analysis of variance F value					
Family (F)	0.0 ns‡	0.5 ns	0.4 ns	0.8 ns	0.1 ns	0.1 ns
Genotype (G)	3.2 ns	4.5 ns	3.1 ns	5.2*	0.6 ns	0.1 ns
F × G	3.7 ns	2.2 ns	0.7 ns	0.0 ns	0.4 ns	0.4 ns

	Total P concentration					
	Break flour			Reduction flour		
Genotype	2003 Aberdeen	2004 Aberdeen	2004 Tetonia	2003 Aberdeen	2004 Aberdeen	2004 Tetonia
	mg g^{-1}					
Intermediate LPA Family						
Wild-type (N = 3)	1.12 ± 0.03	1.03 ± 0.03	0.89 ± 0.02	1.03 ± 0.03	0.89 ± 0.04	0.81 ± 0.01
LPA (N = 5)	1.11 ± 0.02	1.03 ± 0.03	0.93 ± 0.02	1.04 ± 0.02	0.90 ± 0.03	0.82 ± 0.01
High LPA Family						
Wild-type (N = 3)	1.15 ± 0.03	0.98 ± 0.03	0.90 ± 0.02	1.05 ± 0.03	0.88 ± 0.04	0.78 ± 0.02
LPA (N = 3)	1.38 ± 0.03	1.21 ± 0.03	1.03 ± 0.02	1.27 ± 0.03	1.02 ± 0.04	0.95 ± 0.02
	Mixed effects analysis of variance F value					
Family (F)	37.8***	6.6*	9.9**	18.5**	5.5*	11.6**
Genotype (G)	20.8***	21.4***	19.5***	17.0**	8.5*	47.4***
F × G	23.5***	18.7***	7.1*	14.6**	6.9*	34.2***

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.
† LPA, low phytic acid.
‡ ns, nonsignificant.

location as were conducted at Aberdeen, the P concentrations were lower at Tetonia in 2004 than for both years at Aberdeen. Tetonia is approximately 800 m higher in elevation than Aberdeen and has associated with the elevation differences later seeding dates, cooler soil temperatures, and different soil textures. Any of these factors could contribute to P concentration in the seed. However the combination of year and location effect suggests that P concentration can be affected through management and environment.

In contrast to the relatively similar concentrations of total P in bran and shorts, total P concentrations in break and reduction flour fractions were affected by family and genotype in all three environments (Table 1). Moreover, the effect of genotype varied between the two families in all environments. Total P concentrations in break and reduction flour fractions from WT and LPA selections of the Intermediate LPA Family were similar in all three environments. Total P concentrations in break and reduction flour fractions from WT selections of the High LPA Family were similar to those of the WT and LPA selections of the Intermediate LPA Family. However, total P concentrations in reduction flour fractions from LPA selections of the High LPA Family were 0.13 to 0.23 mg g^{-1} greater than in reduction flour fractions from WT selections; similar differences were observed between genotypes in break flour P concentrations (Table 1).

Phytic Acid Phosphorus Concentration in Milling Fractions

Phytic acid P concentration in bran of the LPA selections of the Intermediate LPA Family was reduced by 0.63 to 1.31 mg g^{-1} at Aberdeen, but was not significantly reduced at Tetonia (Table 2). Phytic acid P concentration in bran of LPA selections in the High LPA Family was reduced by 1.74 to 3.09 mg g^{-1} relative to WT selections at Aberdeen, but was not significantly reduced at Tetonia. Similar effects were observed in the shorts fractions in all environments. The percentage of total P accounted for by PAP within WT selections varied among the three environments. In bran from WT selections grown in the Aberdeen trials, PAP accounted for 890 mg g^{-1} of total P in 2003, but only 610 mg g^{-1} of total P in 2004. In bran from WT selections grown in the Tetonia 2004 trial, PAP accounted for 830 mg g^{-1} of total P.

Similar percentage reductions were observed in PAP concentrations in break and reduction flour fractions as were observed in the bran fraction. The effects of the LPA genotype on PAP concentration in break and reduction flour were highly significant in grain grown at Aberdeen. The effect of genotype was nonsignificant, however, in grain produced at Tetonia, which had the lowest PAP concentrations. The absolute magnitude of PAP reductions in break and reduction flour fractions of

Table 2. Phytic acid P concentration in milling fractions of grain grown at Aberdeen, ID, under irrigation in 2003 and 2004 and at Tetonia, ID, under rain-fed conditions in 2004. Concentrations are presented as the mean ± standard error.

	Phytic acid P concentration					
	Bran			Shorts		
Genotype†	2003 Aberdeen	2004 Aberdeen	2004 Tetonia	2003 Aberdeen	2004 Aberdeen	2004 Tetonia
	mg g^{-1}					
Intermediate LPA Family						
Wild-type (N = 3)	9.75 ± 0.39	6.68 ± 0.27	5.01 ± 0.40	4.63 ± 0.17	3.45 ± 0.11	2.33 ± 0.13
LPA (N = 5)	8.44 ± 0.31	6.05 ± 0.21	4.80 ± 0.32	4.16 ± 0.13	3.49 ± 0.09	2.19 ± 0.11
High LPA Family						
Wild-type (N = 3)	9.67 ± 0.39	6.90 ± 0.27	4.74 ± 0.40	4.41 ± 0.17	3.59 ± 0.11	2.17 ± 0.13
LPA (N = 3)	6.58 ± 0.40	5.16 ± 0.27	4.32 ± 0.40	3.27 ± 0.18	3.00 ± 0.11	2.12 ± 0.13
	Mixed effects analysis of variance F value					
Family (F)	6.7*	1.9 ns‡	0.9	11.3**	2.7 ns	1.1
Genotype (G)	34.5***	22.5***	0.6	23.7***	6.8*	0.8
F × G	5.7*	4.9 ns	0.1	4.0 ns	8.6*	0.2

	Phytic acid P concentration					
	Break flour			Reduction flour		
Genotype	2003 Aberdeen	2004 Aberdeen	2004 Tetonia	2003 Aberdeen	2004 Aberdeen	2004 Tetonia
	mg g^{-1}					
Intermediate LPA Family						
Wild-type (N = 3)	0.31 ± 0.01	0.25 ± 0.01	0.20 ± 0.01	0.24 ± 0.01	0.20 ± 0.01	0.13 ± 0.01
LPA (N = 5)	0.27 ± 0.01	0.24 ± 0.01	0.20 ± 0.01	0.22 ± 0.01	0.18 ± 0.01	0.11 ± 0.01
High LPA Family						
Wild-type (N = 3)	0.31 ± 0.01	0.26 ± 0.01	0.21 ± 0.01	0.24 ± 0.01	0.20 ± 0.01	0.11 ± 0.01
LPA (N = 3)	0.24 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.17 ± 0.01	0.14 ± 0.01	0.11 ± 0.01
	Mixed effects analysis of variance F value					
Family (F)	3.5 ns	2.3 ns	1.0	6.4*	4.6 ns	1.9
Genotype (G)	30.6***	11.9**	0.1	27.0***	17.3**	0.2
F × G	2.3 ns	4.1 ns	0.4	5.2*	3.6 ns	1.7

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.
† LPA, low phytic acid.
‡ ns, nonsignificant.

LPA selections grown at Aberdeen was small, as PAP concentrations in flour fractions were about 2% of the concentrations in bran fractions. Febles et al. (2002) determined that PA concentration in factory-made refined flours consumed in the Canary Islands averaged 2.96 mg g^{-1}, which corresponds to 0.83 mg PAP g^{-1} flour, several times greater than the flour concentrations measured in this study. Phytic acid concentration in Pakistani flour (Anjum et al., 2002) ranged from 24 to 48 mg g^{-1}, (corresponding to 6.8 to 12.6 mg g^{-1} PAP), much greater than the estimates of PA concentration in Canary Islands refined flours and those in this study. Le Francois (1988), however, measured PA concentration in flour as 1.30 to 1.83 mg g^{-1}, corresponding to 0.37 to 0.52 mg g^{-1} PAP, closer to the values obtained in this study. These differences may be attributed to differences in the concentration of PAP in the grain being milled and to differences in flour refinement. Therefore the estimates of PAP in the flours of this study are conservative.

Inorganic Phosphorus Concentration in Milling Fractions

Inorganic P concentration in bran of LPA selections of the Intermediate LPA Family was 0.19 to 0.28 mg g^{-1} (22 to 51%) greater than in WT selections (Table 3). And P_i concentration in bran of LPA selections of the High LPA Family was 0.61 to 1.10 mg g^{-1} (100 to 170%) greater than in WT selections. Greater differences between genotypes were observed in grain produced at Aberdeen in 2003 than in 2004; total P concentrations also were greater in 2003 than in 2004.

Differences between genotypes were particularly evident in the reduction flour fractions (Table 3). For example, in 2003 at Aberdeen, the P_i concentration in reduction flour of LPA selections of the High LPA Family was over three times the P_i concentration in reduction flour of WT selections. Differences in P_i concentration between LPA and WT selections of the High LPA Family were greater than differences between LPA and WT selections of the Intermediate LPA Family in all three trials.

Mineral Distribution in Milling Fractions

Mineral concentrations were determined in all milling fractions obtained from the Aberdeen trials in 2003 and 2004. Potassium concentrations in milling fractions were not affected by genotype or family in either year of the study at Aberdeen (data not shown). Similarly, Ca, Fe, Mg, Mn, and Zn concentrations in bran and shorts were not affected by genotype or family in either year (data not shown). In 2004, S and Cu concentrations of bran were affected by genotype (Table 4). Copper and S concentrations in LPA bran in 2004 were 13 and 5% lower,

Table 3. Inorganic P concentration in milling fractions of grain grown at Aberdeen, ID, under irrigation in 2003 and 2004 and at Tetonia, ID, under rain-fed conditions in 2004. Concentrations are presented as the mean ± standard error.

	Inorganic P concentration					
	Bran			Shorts		
Genotype†	2003 Aberdeen	2004 Aberdeen	2004 Tetonia	2003 Aberdeen	2004 Aberdeen	2004 Tetonia
	mg g^{-1}					
Intermediate LPA Family						
Wild-type (N = 3)	0.57 ± 0.11	0.88 ± 0.15	0.60 ± 0.05	0.29 ± 0.08	0.55 ± 0.09	0.35 ± 0.04
LPA (N = 5)	0.85 ± 0.08	1.07 ± 0.12	0.81 ± 0.04	0.41 ± 0.06	0.66 ± 0.08	0.41 ± 0.03
High LPA Family						
Wild-type (N = 3)	0.65 ± 0.11	0.91 ± 0.15	0.60 ± 0.05	0.35 ± 0.08	0.56 ± 0.09	0.34 ± 0.04
LPA (N = 3)	1.75 ± 0.11	1.95 ± 0.15	1.21 ± 0.05	0.91 ± 0.08	0.96 ± 0.09	0.57 ± 0.04
	Mixed effects analysis of variance F value					
Family (F)	22.6***	10.7**	20.6***	13.8**	4.1 ns‡	6.6**
Genotype (G)	45.4***	19.5***	81.5***	19.9**	11.0**	25.4***
F × G	15.6**	9.1**	19.6***	8.5*	3.5 ns	8.1**

	Inorganic P concentration					
	Break flour			Reduction flour		
Genotype	2003 Aberdeen	2004 Aberdeen	2004 Tetonia	2003 Aberdeen	2004 Aberdeen	2004 Tetonia
	mg g^{-1}					
Intermediate LPA Family						
Wild-type (N = 3)	0.05 ± 0.01	0.08 ± 0.03	0.07 ± 0.01	0.05 ± 0.02	0.07 ± 0.03	0.05 ± 0.01
LPA (N = 5)	0.08 ± 0.01	0.11 ± 0.02	0.10 ± 0.01	0.09 ± 0.02	0.10 ± 0.03	0.08 ± 0.01
High LPA Family						
Wild-type (N = 3)	0.07 ± 0.01	0.09 ± 0.03	0.08 ± 0.01	0.06 ± 0.02	0.07 ± 0.03	0.06 ± 0.01
LPA (N = 3)	0.22 ± 0.01	0.27 ± 0.03	0.18 ± 0.01	0.27 ± 0.02	0.27 ± 0.03	0.17 ± 0.01
	Mixed effects analysis of variance F value					
Family (F)	38.6***	10.7**	39.7***	27.8***	8.9**	30.1***
Genotype (G)	44.7***	18.1***	98.8***	41.9***	15.3**	73.8***
F × G	20.8***	8.6*	28.3***	20.4***	8.0*	22.8***

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.
† LPA, low phytic acid.
‡ ns, nonsignificant.

Table 4. Effect of family and genotype on Cu, S, Ca, and Fe concentration in milling fractions of grain grown at Aberdeen, ID, in 2003 and 2004. Concentrations are presented as the mean ± standard error.

	Cu		S		Ca				Fe			
	Bran				Break flour		Reduction flour		Break flour		Reduction flour	
	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
	Mixed effects analysis of variance *F* value											
Family (F)	1.1 ns†	0.5 ns	0.4 ns	0.0 ns	0.4 ns	0.0 ns	2.2 ns	0.1 ns	0.9 ns	0.1 ns	0.0 ns	1.3 ns
Genotype (G)	1.3 ns	19.2**	1.8 ns	10.7**	0.6 ns	6.7*	0.4 ns	9.1*	0.0 ns	5.5*	0.6 ns	10.8**
F × G	0.7 ns	0.2 ns	1.0 ns	0.1 ns	0.1 ns	0.1 ns	0.3 ns	0.3 ns	4.5 ns	0.5 ns	0.2 ns	0.0 ns
	mg kg^{-1}											
Genotype												
Wild-type	15.4 ± 0.8	13.2 ± 0.4	2410 ± 110	2110 ± 25	203 ± 9	207 ± 5	179 ± 8	178 ± 5	6.25 ± 0.22	6.33 ± 0.21	5.87 ± 0.59	5.28 ± 0.12
LPA‡	14.7 ± 0.8	11.5 ± 0.4	2300 ± 100	2000 ± 20	211 ± 8	219 ± 5	185 ± 8	193 ± 5	6.22 ± 0.20	5.72 ± 0.19	6.36 ± 0.56	4.76 ± 0.11

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
† ns, nonsignificant.
‡ LPA, low phytic acid.

respectively, than in WT bran. In 2004, S concentration in LPA and WT break flours were 1560 and 1670 mg kg^{-1}, respectively (genotype *F* value 14.8, $P < 0.01$).

Small, but statistically significant, effects of LPA genotype on mineral concentrations were observed in flour fractions in 2004. For example, in 2004, concentrations of Ca and Fe in break and reduction flour were affected by genotype (Table 4). In 2004, Ca concentration in LPA genotype break and reduction flours was 6 to 8% greater than concentration in WT flour fractions. In contrast, Fe concentration in LPA genotype break and reduction flours was 10% lower than in WT flours in 2004. That similar effects were observed in both break and reduction flour fractions suggests that these observations are not a result of Type I error. In 2003, concentration of Mn in both break and reduction flour was greater in LPA selections than in WT selections [genotype *F* values 6.6 and 5.9 ($P < 0.05$), respectively]. Manganese concentrations in LPA and WT break flours in 2003 were 6.8 and 6.0 mg kg^{-1}, respectively; Mn concentrations in LPA and WT reduction flours in 2003 were 6.8 and 5.9 mg kg^{-1}, respectively.

The most consistent effects of the LPA genotype and family were observed on Mg concentration in break and reduction flour fractions. In both 2003 and 2004 trials grown at Aberdeen, Mg concentration was greater in both break and reduction flour of LPA selections than in the WT selections of the High LPA Family. On average, Mg concentration was 51 mg kg^{-1} greater in reduction flour of LPA selections than of WT selections (Table 5). Magnesium concentration also was 77 mg kg^{-1} (21%) greater in break flour of LPA selections of the High LPA Family grown at Tetonia in 2004 than in WT selections (Table 6). The only other significant effect of genotype in selections from the High LPA Family at Tetonia was a 110 mg kg^{-1} (10%) increase in K concentration in LPA selections relative to WT selections (Table 6). Effects of the LPA genotype on Mg distribution may be greater

Table 5. Magnesium concentration in flour fractions of LPA and WT grain grown at Aberdeen, ID, under irrigation in 2003 and 2004. Concentrations are presented as the mean ± standard error.

	Mg concentration			
	Break flour		Reduction flour	
Genotype†	2003	2004	2003	2004
	mg kg^{-1}			
Intermediate LPA Family				
Wild-type	389 ± 19	334 ± 10	338 ± 18	279 ± 8
LPA	380 ± 16	351 ± 8	352 ± 15	307 ± 7
High LPA Family				
Wild-type	344 ± 19	318 ± 10	281 ± 18	259 ± 8
LPA	429 ± 20	376 ± 10	371 ± 19	322 ± 8
	Mixed effects analysis of variance *F* value			
Effect				
Family (F)	0.0 ns‡	0.2 ns	1.4 ns	0.1 ns
Genotype (G)	6.8*	15.3**	11.0**	40.6***
F × G	10.3**	4.8 ns	5.9*	6.2*

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.
† LPA, low phytic acid.
‡ ns, nonsignificant.

Table 6. Magnesium and K concentration in break flour of the High LPA Family low phytic acid (LPA) and wild-type (WT) selections grown at Tetonia, ID, in 2004. Concentrations are presented as the mean ± standard error.

Genotype	Mg	K
	mg kg^{-1}	
WT	363 ± 13	997 ± 45
LPA	439 ± 13	1107 ± 45
	Mixed effects analysis of variance *F* value (genotype)	
	17.7***	4.7*

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

than effects on distribution of other minerals because of the relative solubility of Mg cations. Moreover, Ca and K cation concentrations are actively regulated in cells.

CONCLUSIONS

The decrease in PAP and increase in P_i observed in bran fractions in LPA genotypes was anticipated based on the localization of PA in the aleurone. The increase in total P concentration in the break flour and reduction flour fractions of LPA selections of the High LPA Family is consistent with our previous report (Guttieri et al., 2004) and results with the *lpa*1–1 mutant of rice (Bryant et al., 2005). The distribution of P in break and reduction flour fractions of LPA selections also is shifted from PAP toward P_i. The greater effect of genotype in 2003 suggests an effect of available soil P, an observation that merits further investigation.

In the earlier report (Guttieri et al., 2004), our formal segregation analysis concluded that the LPA phenotype was conditioned by two or more independently segregating genes. During the derivation of the backcross families for this study we stabilized lines that were homogeneous for the HIP phenotype in the seed. The two families produced phenotypes that were significantly different in the level of the LPA phenotype. The use of backcrossing should minimize background effects, therefore the most conservative conclusion from these observations suggest that the High LPA Family has either two LPA genes in contrast to a single gene in the Intermediate LPA Family, or that the High LPA Family has a gene conferring a greater reduction in PA than the more moderate effect of the Intermediate LPA Family gene. Many of the family interactions observed in this study are consistent with a genetically conferred stronger expression of the LPA trait in the High LPA Family than in the Intermediate LPA Family.

Effects of the LPA trait on other mineral concentrations in milling fractions were limited. The most generalized effect of the LPA mutant was observed in Mg concentrations in flour fractions, which averaged 25% greater in LPA selections of the High LPA Family than in WT selections. Effects on mineral concentrations other than Mg and P were not consistent across environments, suggesting that such effects, observed in only one environment, may not be general. For animal rations the alteration in P availability may be very significant and warrants feeding studies to confirm its relative utility. Magnesium deficiency is well documented within North America and has been linked with osteoporosis (reviewed by Rude and Gruber, 2004), as well as with insulin resistance and increased risk for Type II diabetes (Huerta et al., 2005). The shift in Mg within the milling streams of LPA wheat warrants further investigation into its biological significance.

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